REMARKS

Claims 1, 2, 4-19, and 21-55 are pending and have been rejected. Claims 15, 28, and 30-37 are withdrawn from consideration. Claims 39, 42, 45, 48 and 51 have been amended. Claims 1, 2, 4-19, and 21-55 remain in the case.

The examiner again notes that applicant did not traverse the restriction requirement, and applicants reiterate that elected Group I includes product claims, and that once the elected product claims are found allowable, method claims 15, 28 and 30-37 should be rejoined and examined.

A rejection of claims 1-14 and 16-18 under the second paragraph of Section 112 has been withdrawn. Rejections under Section 102 based on Sivam or Leung *et al.*, and rejections under Section 103 based on Sivam in view of Hansen and Sivam in view of Li *et al.* have been withdrawn. A rejection for obviousness-type double patenting based on U.S. 5,443,953 also has been withdrawn.

Claims 1, 4-14, 16-18, 38, 39, 41-42, 44, 45, 47, 48, 50, 51 and 55 are rejected under the second paragraph of Section 112. The examiner maintains his position that the phrase "ketone derivative of a saccharide or saccharide precursor" is indefinite, urging that the exact meaning of the phrase is unclear because there is no universally accepted meaning for this term and it is not adequately defined in the specification.

The present invention relates to glycosylated antibodies, and this context provides the skilled artisan with guidance on the scope of the terms questioned by the examiner. There are a very limited number of saccharides that are used in glycosylation. Appended to this response is an excerpt from Stryer, Biochemistry. Figs. 14-18 show the formulae of saccharides commonly found in oligosaccharide units of glycoproteins; these include β-L-fucose, β-D-galactose, β-D-N-acetylgalactosamine, β-D-N-acetylglucosamine, β-D-mannose, and sialic acid.

The examiner finds the term "saccharide precursor" to be unclear, and wonders whether the phrase means a molecule that can be used in the biosynthetic pathway to a saccharide such as starch, carbon atoms, etc." Taken in the context of glycosylation, however, it is abundantly clear to the skilled artisan that this term refers to molecules that

can be converted by biosynthetic pathways in the cell to one of the limited number of saccharides that are used in glycosylation. The examiner is again referred to the disclosure on page 7 of the specification of

> a ketone derivative of a saccharide (such as N-levulinoyl fucose) or saccharide precursor (such as N-levulinoyl mannosamine (ManLev)), resulting in an antibody comprising reactive ketone groups at the N-glycosylation sites. In the case of ManLev, biosynthetic pathways convert the ManLev to levulinoyl sialic acid, which is incorporated into the antibody at the glycosylation site. In the case of N-levulinoyl fucose, the N-levulinoyl fucose itself is incorporated into the antibody at the glycosylation site.

Thus, a saccharide precursor is a precursor of one of the saccharides used in glycosylation of glycoproteins, which is converted by the cell's biosynthetic pathways into a saccharide that is incorporated into a glycoprotein.

There are a limited number of saccharide precursors. For example, the specification mentions mannosamine, which can be converted by the cell's biosynthetic machinery to sialic acid, one of the common saccharides used in glycosylation. Mannosamine is one of several well-known saccharide precursors, and the terminology saccharide precursor typically is used in this field. For example, Yarema *et al.*, abstract appended, describe "[delivery of] a uniquely reactive ketone group to endogenous cell surface sialic acid residues by treating cells with ***the ketone-bearing metabolic precursor N-levulinoylmannosamine (ManLev).***"

The examiner also questions the term ketone derivative. In particular, he questions whether the phrase "ketone derivative" means an aldehyde. It does not, and there would be no reason for such an assumption. Moreover, a skilled artisan clearly would be apprised that a "ketone derivative" refers to compounds in which a ketone functionality is introduced on a saccharide, for example, as an N-acyl group such as levulinoyl. For example, the specification shows the saccharide N-levulinoyl fucose, and the saccharide precursor N-levulinoyl mannosamine (ManLev), both of which are "ketone derivatives" which have a ketone functionality on the N-acyl levulinoyl group. A skilled artisan clearly would be apprised that a "ketone derivative" refers to a saccharide with an N-acyl group that contains a ketone function, such as levulinoyl.

Based on the foregoing, reconsideration and withdrawal of this basis for rejection under the second paragraph of Section 112 is respectfully requested.

Claims 1, 2, 4-14, 16-19, 21-27, 29, 38, 41, 44, 47, 50, and 53-55 are rejected under the first paragraph of Section 112. The examiner refers to disclosure in the specification that "not all engineered sites are glycosylated." In particular, he notes that computer modeling "was used in the instant application for those sites in the Cκ sites and in the KCN1-5 sites these sites were not glycosylated." The fact that every site identified by computer modeling as a potential glycosylation site is not, in practice, glycosylated does not, however, lead to a conclusion that the present claims are not enabled. The approach described in the specification clearly is a screening approach, in which a number of potential glycosylation sites are designed based on the guidance described in Example 2 and computer modeling, and then those sites which actually are glycosylated when the IgG is expressed in mammalian cells are subsequently identified. As stated in a review by Kornfeld & Kornfeld (*Ann. Rev. Biochem.* 1985, 54:631-664, "An examination of protein sequences has revealed that only about one third of the potential Asn-X-Ser/Thr sites in proteins are actually glycosylated" (citing work by Kronquist and Lennarz, *J. Supramol. Struct.* 1978, 8:51-65). Thus, the results reported in the present specification are in line with those which would have been expected by a person of ordinary skill in the art. The present specification enables a skilled artisan to identify potential glycosylation sites, and then identify those sites which actually are glycosylated *in vivo*. Reconsideration and withdrawal of the rejection under the first paragraph of Section 112 is respectfully requested.

Claims 39, 42, 45, 48, and 51 are rejected under the second paragraph of Section 112, and have been amended for clarity.

Claims 39, 40, 42,43, 45, 46, 48, 49, 51 and 52 are rejected under the first paragraph of Section 112. The examiner states that it is unknown whether cell lines that produce an antibody having the exact chemical identity of hLL2HCN1, hLL2HCN5 and hLL2Vκ are known and publicly available, or can be reproducibly isolated without undue experimentation. A cell line that produces such an antibody need not be known or publicly available, as the present specification clearly enables a skilled artisan to produce such a cell

line *de novo*. The sequence of the hLL2 antibody is known,[1] and the design of and testing of hLL2 antibodies with HCN1, HCN5 and Vκ N-glycosylated sites in hLL2 is clearly described. The making of a cell line which expresses a vector that produces such antibodies is straightforward -- applicants exemplify transfection of SP2/0 cells, and the presently rejected claims all recite transfecting SP2/0 cells with a vector encoding an antibody having a HCN1, HCN5 or Vκ N-glycosylation site which is selected from the group consisting of hLL2HCN1, hLL2HCN5 and hLL2Vκ-N. Moreover, sequences of hLL2HCN1 and hLL2HCN5 are described in U.S. 6,254,868. Reconsideration and withdrawal of this rejection under the first paragraph of Section 112 is respectfully requested.

Claims 19-26 and 53-55 are rejected under Section 103(a) based on Shih *et al.* in view of Leung *et al.* and Qu *et al.* The examiner urges that Shih *et al.* disclose oxidizing a carbohydrate of an antibody to produce ketones and conjugating drugs and toxins to the oxidized antibody. The examiner admits that Shih does not teach glycosylation at the HCN1, HCN5 or Vκ-N site, but alleges that these deficiencies are made up for by the teaching of Leung and Qu.

The portion of Shih that is cited by the examiner actually is a reference to a published application by McKearn (EP 88,695), which discloses "a method for preparing antibody conjugates which involves oxidizing the carbohydrate portion of the antibody and linking compounds with free amine groups to the resultant carbonyls (aldehyde and/or ketone groups) by Schiff base formation. The harsh oxidation used to open the ring and thereby generate the carbonyl groups, especially where complete oxidation of all carbohydrate residues is desired, and the harsh reducing environment used to stabilize the Schiff base conjugate, both may impair the biological activity of the molecule. By contrast, the antibodies according to the present invention already have a reactive ketone group as a side chain on the carbohydrate used in the glycosylation of the antibody, which is produced by the transfected host cell's biosynthetic machinery. Shih (McKearn) does not disclose a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site that is not introduced by harsh oxidation. In particular, Shih

[1] See, for example, U.S. 5,789,554.

(McKearn) does not disclose such a glycosylated antibody prepared by the method of claim 1.

The addition of Leung and/or Qu to Shih (McKearn) would not have suggested the invention as presently claimed. Leung *et al* discloses a glycosylation site in the Vκ domain and that this site can be used for conjugations. Leung *et al.* references other articles which disclose the conjugation technique. Like Shih (McKearn), these entail chemical oxidation of the rings to generate reactive aldehyde groups, which then can be covalently bonded to the amino groups of chelates or drugs through Schiff bases. Since only the C-C bonds with hydroxyl groups attached to each carbon can be periodate-oxidized to form two aldehyde groups, the maximum number of these reactive sites is dictated by the structure and linkages of the oligosaccharide, hence Leung's disclosure that an average of 2 to 6 chelators such as DTPA could be conjugated..

Qu *et al.*, *Glycobiol.* 7(6): 803-09 (1997) teach the compositions and sequences of CH1-appended carbohydrates from two antibodies, hLL2HCN1 and hLL2HCN5, as determined by fluorophore-assisted carbohydrate electrophoresis (FACE). The structural profile of hLL2HCN1-carbohydrates revealed that about 2-4 hexose rings in an oligosaccharide chain are available for periodate oxidation. Therefore, a maximum of 8-16 aldehyde groups on average can be generated from the carbohydrate side chains of each hLL2HCN1 F(ab')2 fragment. With the average size of hLL2HCN5-carbohydrate being 3-4 monosaccharide residues larger than that of HCN1, a higher number of maximum achievable aldehyde groups for hLL2HCN5 is expected.

Qu *et al.* does not overcome Shih's failure to teach conjugation methods that use introduced reactive ketone groups on the side chains of the glycosylation carbohydrates, as opposed to chemical oxidation of the carbohydrate ring and subsequent covalent bonding of the thus-generated aldehyde groups to the amino groups of chelates or drugs through Schiff bases. Since only the C-C bonds with hydroxyl groups attached to each carbon can be periodate-oxidized to form two aldehyde groups, the maximum number of these reactive sites is dictated by the structure and linkages of the oligosaccharide. As discussed above, chemical oxidation to generate carbonyl groups has significant adverse consequences. When harsh conditions are used to generate the maximum number of such groups, the

three-dimensional structure of the antibodies is altered and the immunoreactivities of the antibodies may suffer. And under milder chemical conditions, only 1.6 and 3 molecules of DTPA are conjugated to the F(ab')2 of hLL2HCN1 and hLL2HCN5 sites, respectively, probably due to inefficient oxidation of hexose rings under these conditions.

All of the references cited by the examiner disclose the use of harsh oxidation conditions to derivatize a glycosylated antibody. The antibodies according to the present invention, on the other hand, have a reactive ketone group on a side chain, and are produced by the transfected host cell's biosynthetic machinery. None of the cited references disclose or suggest a glycosylated antibody or antigen-binding antibody fragment having a reactive ketone group on the glycosylated site, and more particularly a glycosylated antibody prepared by the method of claim 1. In accordance with the present invention, these antibodies are made recombinantly by a transfected host cell. The host cell's biosynthetic machinery converts the antibodies so that they have a reactive ketone group. Reconsideration and withdrawal of the rejections under Sections 102 and 103 based on Shih, Leung, and Qu is respectfully requested.

Based on the foregoing amendments and remarks, all claims are believed to be in condition for allowance. Should there be any matter requiring further attention, the examiner is invited to contact the undersigned at the local telephone exchange listed below.

Respectfully submitted,

December 21, 2001
Date

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FOLEY & LARDNER

MARKED-UP VERSIONS OF CLAIM AMENDMENTS

39. (Amended) A method according to claim 38, wherein said antibody [is] having a HCN1, HCN5 or Vκ N-glycosylation site is selected from the group consisting of hLL2HCN1, hLL2HCN5 [or] and hLL2Vκ-N.

42. (Amended) A method according to claim 41, wherein said antibody [is] having a HCN1, HCN5 or Vκ N-glycosylation site is selected from the group consisting of hLL2HCN1, hLL2HCN5 [or] and hLL2Vκ-N.

45. (Amended) A method according to claim 44, wherein said antibody [is] having a HCN1, HCN5 or Vκ N-glycosylation site is selected from the group consisting of hLL2HCN1, hLL2HCN5 [or] and hLL2Vκ-N.

48. (Amended) A method according to claim 47, wherein said antibody [is] having a HCN1, HCN5 or Vκ N-glycosylation site is selected from the group consisting of hLL2HCN1, hLL2HCN5 [or] and hLL2Vκ-N.

51. (Amended) A method according to claim 50, wherein said antibody [is] having a HCN1, HCN5 or Vκ N-glycosylation site is selected from the group consisting of hLL2HCN1, hLL2HCN5 [or] and hLL2Vκ-N.